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EVALUATION OF FUNGAL SPECIES ASSOCIATED WITH DRIED OGI

U. Ajima[1], A.I. Ogbonna[2], P.N. Olotu[3] , A.U. Asuke[4]
[1]Department of Pharmaceutical Chemistry, [2]Department of Microbiology, [3]Department of Pharmacognosy, University of Jos, Jos, Nigeria, [4]Department of Microbiology, Ahmadu Bello University Zaria, Nigeria.

ABSTRACT
Ogi is a fermented maize product usually used for weaning children in parts of Southern Nigeria. It is sometimes processed by sun drying to preserve and increase its shelf life. This study involves an investigation on the species of fungi associated with dried Ogi.. Soil-Plate isolation method was employed to isolate the fungal species on two conventional fungal culture media (Sabouraud Dextrose Agar and Potato Dextrose Agar). The experimental dried Ogi was assessed for its pH and percentage moisture content. A total of 10 species of fungi belonging to 8 genera were isolated. Three (3) species of *Aspergillus* were isolated with *Aspergillus niger* having the highest frequency of occurrence of 17.65%, which was the same with the genera *Penicillium* and *Saccharomyces.* The pH range of the dried Ogi was within the range that supports fungi growth in culture. The significance of fungi in Ogi with regards to its Clinical and Industrial relevance has also been discussed.

KEYWORDS: Fungi, Dried Ogi, Preservation, Fermented cereal products, Weaning foods.

INTRODUCTION

Ogi is a staple cereal fermentation product found predominantly in Southern Nigeria and is the first native food given to babies at weaning. It is produced generally by soaking maize grains in warm water for 2-3 days followed by wet milling and sieving through a screen mesh. The sieved material is allowed to sediment and ferment and is marketed as wet cakes wrapped in leaves (Jay, 2004). Nnanyelugo and Onofiok (2004) reported the use of Ogi as a weaning food in Southern Nigeria to supplement breastfeeding, which may be inadequate to meet the nutritional demands of the growing infant. The same authors also reported that Ogi is usually introduced to the infant between the ages of 3-6 months. It has also been shown that Ogi Liquor has both antibacterial (Adebolu *et al*, 2007) and antifungal properties (Adebayo and Aderiye, 2010).

The importance of Ogi to the food economy of rural communities in Southern Nigeria cannot be overemphasized. Aderiye and Laleye (2003) in a study in Southwest Nigeria discovered that garri and Ogi are the most frequently consumed fermented foods in that area. Also, Ajayi (2004) showed that cereal grain processing industries, using maize as their raw material to produce ogi, constituted about 11.19% of the total food processing industries in rural Oyo State, Nigeria. However, the fermented foods of Nigeria are at present mostly produced on a small scale, household basis under highly variable conditions, which result in food of unpredictable quality. The fermentation techniques are often characterized by the use of simple non-sterile equipment, chance or natural inoculum, unregulated conditions, sensory fluctuations, poor durability and unattractive packing of the product (Anonymous, 1973). With increasing industrialization and urbanization, efforts are presently geared towards the development of large-scale factory production facilities for these foods where the quality of the finished product will be assured.

Botanically, *Zea mays* belongs to the grass Family *Gramineae* and is a tall annual plant with an extensive fibrous root system. It is a cross pollinating specie with the female (ear) and male (tassel) flowers in separate places on the plant. The grain develops in the ears or cobs, often one on each stalk. The kernels are often white or yellow in colour although black, red and a mixture of colours are also found (FAO, 1992).

Fermentation is the anaerobic breakdown of an organic substance by an enzyme system, in which the final hydrogen acceptor is an organic compound (Banwart, 1974). Fermentation may be by yeasts, moulds, bacteria or combinations of these organisms (Frazier and Westhoff, 1988). Numerous food products owe their production and characteristics to the activities of microorganisms. Some advantages of food fermentation include: general improvement in the shelf life, texture, taste and aroma, nutritional value and digestibility and it leads to significant lowering the content of anti-nutrients of cereal products. (Kohajdova and Karovicova, 2007).

Fungi are nucleated, spore-bearing micro-organisms which do not possess chlorophyll, generally reproduce both sexually and asexually and have somatic structures which are surrounded by cell walls consisting of polysaccharides, cellulose and/ or chitin, mannan or glucan (Hugo and Russell, 1998). Fungi may be multicellular or unicellular (Banwart, 1974). Moulds belong to the Eumycetes or True fungi and are multicellular filamentous fungi whose growth on foods is readily recognized by its fuzzy appearance. Yeasts on the other hand are unicellular fungi, which are able to reproduce vegetatively by means of simple cells, which bud or less commonly, divide by fission (Pitt and Hocking, 1997). Yeasts differ from most fungi, which grow as thread-like hyphae, but this differentiation is not a fundamental one because some fungi can alternate between yeasts and hyphal phase depending on environmental conditions. Such fungi are termed dimorphic, (Deacon, 2005).

According to Frazier and Westhoff (1988), the exterior of harvested grains retains some of the natural flora they had while growing. Other sources of organisms on these grains include soil, insects etc. Banwart (1974) reported that the bacterial flora on maize may include families like *Pseudomonaceae, Micrococcaceae, Lactobacillaceae* and *Bacillaceae,* while moulds such as *Aspergillus sp., Fusarium sp. and Penicillium sp.* are found on dry products such as grains and peas.

Jay (2004) reported that *Corynebacterium sp., S. cerevisiae, Zygosaccharomyces rouxii* and the lactic acid bacteria such as *L. plantarum and L. lactis* are prominent in the traditional ogi fermentation, as are the fungi *Cephalosprium sp., Fusarium sp., Aspergillus sp.* and *Penicillium sp.* The changes occurring during the spoilage of Ogi were studied by Odunfa and Teniola (2002) and Omemu *et al* (2007) and they identified the following organisms as been responsible for its spoilage: Moulds; *Aspergillus niger, A. flavus, Penicillium sp.,* Yeast isolates; *Geotrichum candidum, Candida krusei, and C. valida, Sacharromyces cerevisiae,* while the bacteria include: *L. brevis, L. plantarum, Bacillus subtilis, Pediococcus pentosaceus, Brevibacterium linens and Br. Oxidans.* In a study on the evaluation of the hazards and critical control points of Ogi small scale processing centers in Abeokuta, Nigeria (Omemu and Adeosun, 2010) similar organisms as above were isolated as some of the microbial contaminants of Ogi with the Raw materials, steeping and fermentation identified as the critical control points.

Akinrele (1970) reported that Ogi contains Riboflavin, Niacin, Thiamine and several amino acids by virtue of the fermentation process. Also, Akinrele (1970) and Banigo and Muller (1972) reported on the carboxylic acids in Ogi and found Lactic acid in greatest concentration (0.55%) followed by Acetic acid (0.09%) and smaller amounts of Butyric acid (Acetic acid is responsible for its sour taste). These authors also reported on amino acid content and found no difference between maize flour and Ogi for all amino acids including the essential ones. The ogi samples however had twice the amount of Serine and somewhat higher values for Glutamic acid. Adeniji and Potter (1978) reported that Ogi processing did not decrease protein content of maize but total and available Lysine was significantly decreased. On the other hand, Tryptophan levels were more stable. These authors also found an increase in neutral detergent fiber and ash but no change in lignin.

TABLE 1: NUTRITIVE VALUE OF SOME TRADITIONAL WEANING FOODS AS COMPARED TO COMMERCIAL WEANING PRODUCTS.

Food	Energy (Kcal)	Ash ($_g$/100$_g$ Dry weight)	Protein (g/100g Dry weight)	Carbohydrates (g/100g dry weight)
Traditional Weaning Foods				
1. Maize Pap (ogi)	417	0.2	6	91
2.Guineaconvpap	415	0.5	4	92
3 Millet Pap	419	0.5	7	88
4.Guineacorn Porridge	412	1.0	5	91
Commercial Products				
l.Cerelac®	412	3.3	16	67
2.Lactogen®	463	4.8	22	52

Source: Nnanyelugo an Onofiok, 2004.

As can be seen from Table 1 above, traditional weaning foods in West Africa are of low nutritive value and are characterized by low protein, low energy density and high bulk. Ogi has been implicated in the aetiology of Protein-Energy Malnutrition in children during the weaning period (Naismith, 1973) and if severe it can result in kwashiorkor and marasmus. The nutritive value of Ogi has been improved by combining it with locally available foods that supplement it with nutrients in which it is deficient (Fashakin and Ogunsoola, 1982; Akinrele and Edwards, 1971; Aminigo and Akingbala, 2004; Aminigo and Ossai, 1999; Ekpenyong *et al*, 1997).

Sun drying of food has been shown to remove water, reduce moisture content and concentrate nutrients (Baiyeri, 2004). Some consumers of Ogi dry it after preparation in order to elongate its shelf life. In the rural setting, this is usually done by sun drying in the open and this may lead to the introduction of some fungal contaminants and their spores into the Ogi. Some of these fungi may be capable of producing mycotoxins.

Mycotoxins are secondary metabolites of certain filamentous fungi that can cause a wide spectrum of toxicological effects in both humans and animals when low concentrations are ingested or inhaled (Smith *et al*., 1995). Almost all fermented foods and beverages have the potential to be contaminated by toxigenic moulds at some stage during their production, processing, transport or storage (Katongole, 2008). Mycotoxins are a structurally diverse group of mainly small molecular weight compounds produced mostly by five genera of fungi, *viz*: *Aspergillus, Penicillium, Fusarium, Alternaria* and *Claviceps* (Smith and Moss, 1985). Some of the most widely reported mycotoxins produced by the five genera include: *Aspergillus* toxins: aflatoxins B1, G1 and M1, ochratoxin A, sterigmatocystin; *Penicillium* toxins: cyclopiazonic acid, citrinin and patulin; *Fusarium* toxins: deoxynivalenol, nivalenol and fumonisins; *Alternaria* toxins: tenuazonic acid, alternariol and alternariol methyl ether; *Claviceps* toxins: ergot alkaloids (Steyn, 1995). Some mycotoxins have also been shown to be mutagenic and carcinogenic while others show specific organ toxicity. Some fungi are pathogenic and can cause systemic illness e.g. *'Candida sp.* is implicated in Candidiasis. Also, the sporogenic fungi, when ingested in the Ogi may come out of sporulation to cause illness.

Hence, because of its widespread consumption in the Country, it is important to analyze dried Ogi to detect any contaminants, which may present a health hazard to the consumers especially the growing infant, so that appropriate measure can be taken. The study therefore aims at detecting the presence of fungal contaminants in dried Ogi which may be present as a result of the drying process or which may have been introduced during processing of the gruel or from various other sources. This is to be achieved by isolating and identifying these fungal species.

MATERIALS AND METHODS

MATERIALS

Sabouraud Dextrose Agar (SDA) and Potato Dextrose Agar (PDA) were manufactured by Fluka.

SAMPLE COLLECTION

The maize grains used in preparing the Ogi were *Zea mays L.* Family *Gramineae.* Collection of the grains was done at Terminus market in Jos Metropolis.

PRODUCTION OF THE OGI

The Ogi preparation method employed in this study is as described by Adegoke and Babalola (1988) with slight modification. The procedure consists of several steps as shown in Figure 1 below.

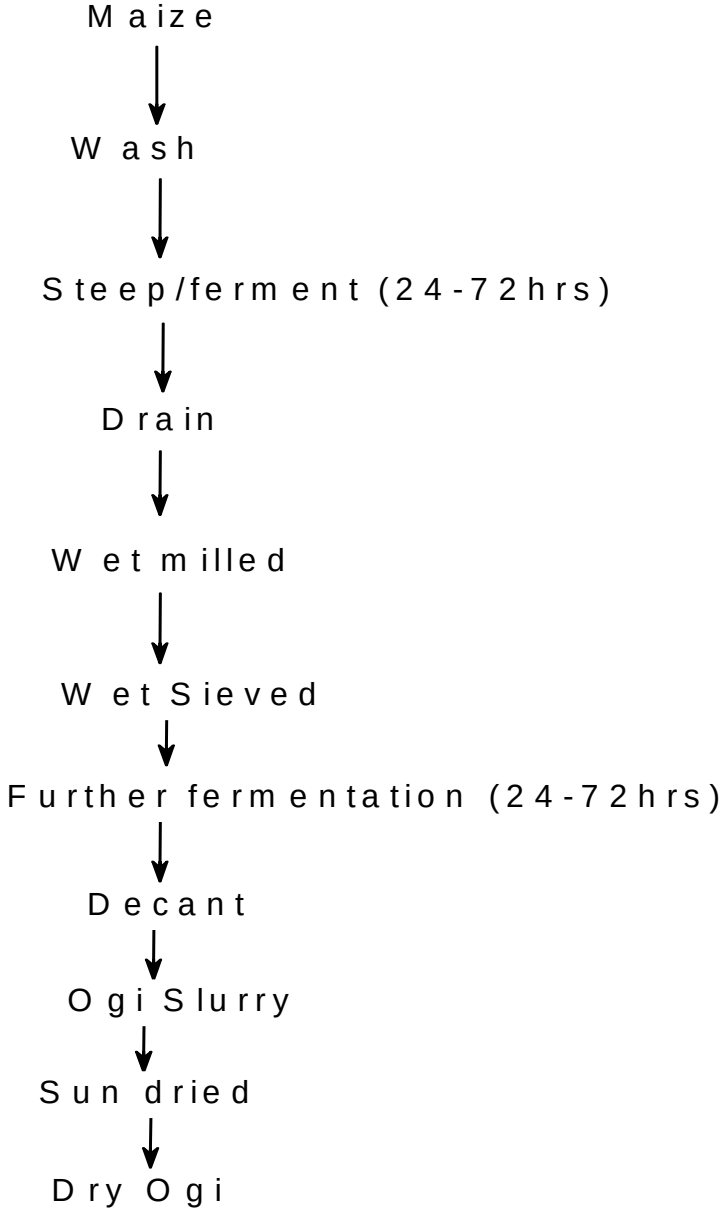

Figure 1: Traditional process for producing dried Ogi

The Ogi after drying was in mounds and these were then collected aseptically and the mounds were then triturated in a sterile porcelain mortar until a smooth powder was obtained and this was then transferred aseptically into a sterile container and kept for use in the work.

ISOLATION OF FUNGAL SPECIES FROM THE DRIED OGI

Eight (8) Petri dishes were employed in the study. Three (3) Petri dishes each for the 2 media (i.e. SDA and PDA) were prepared, together with one control plate in each group. A 0.5ml of 3mg/ml Streptomycin was incorporated into each plate to inhibit bacterial growth. The Soil-Plate method as described by Okonkwoeje (2000) was used to inoculate the dried ogi. It involved sprinkling about 0.2g of the dried ogi powder unto each plate before pouring 20ml of the prepared media and swirling the plate around gently before allowing to set. Each batch of 4 plates including the control were then incubated at 25°C for 7 days. The culture plates were then examined for the presence of growth. The different growths obtained were then subcultured severally unto fresh culture plates until pure, cultures were obtained. The above procedure was again repeated but this time around the plates were incubated at 37°C for 7days.

IDENTIFICATION OF THE FUNGAL ISOLATES

The identification of the fungal species was carried out using a number of observations such as macroscopic studies, Microscopic examination, Morphology (size and shape) and also using gram staining characteristics for yeasts as, described by Cheesebrough (2000). Slides of the fungal isolates were then prepared and observed under the microscope and the organisms identified on the basis of their appearance with reference to Webster (1983) and Samson *et.al* (1984). Photomicrograghs of some of the slides were then taken.

pH DETERMINATION OF THE OGI SAMPLE

5g of the dried ogi sample was weighed and dissolved in 20ml of sterile distilled water and the slurry obtained was then placed in a beaker and its pH taken using a pH meter. The pH was taken 3 times and the average pH calculated and recorded. The pH meter was first calibrated with a buffer solution before it was used to determine the pH of the Ogi.

PHYSICAL EXAMINATION OF THE OGI

The dried Ogi sample was examined physically to observe its ecological characteristics such as colour, odour and texture.

DETERMINATION OF WATER CONTENT

The percentage moisture content of the ogi sample was determined by drying the Ogi to constant weight in an oven with a temperature of 105°C. The % moisture content of the, Ogi was then determined using the following formula;

If the weight of the original Ogi sample = Wg
If the weight of the Ogi after drying to constant weight = Xg
Then, the moisture content of the ogi sample = (W - X)g
% Moisture content of Ogi sample = $\frac{W - X}{W} \times lOOg$

The result is as presented in Table 3.

RESULTS

The results obtained from this study reveal that a variety of fungal species belonging to different genera are associated with dried Ogi. A total of 10 species of fungi belonging to 8 different genera were isolated from the dried Ogi on the different media used as illustrated in Table 2.

TABLE 2: FUNGAL SPECIES ISOLATED FROM DRIED OGI USING TWO DIFFERENT MEDIA.

	Media Used For Isolation	
FUNGAL SPECIE	PDA	SDA
1. *Alternaria sp.*	-	-
2. Aspergillus niger	+	+
3. *A.fiavus*	-	+
4. *A. fumigates*	+	+
5. *Cladosporium sp.*	-	-
6. *Curvularia sp.*	+	-
7. *Fusarium sp.*	+	-
8. *Mucor sp.*	_	+
9. *Penicillium sp.*	+	+
10. *Saccharomyces sp.*	+	+

Key: + = Present, - = Absent, PDA = Potato dextrose agar SDA = Sabouraud dextrose agar

Among the species of fungi isolated, it was observed that their distribution on the media used varied. For instance, *Aspergillus niger, Penicillium sp.* and *Saccharomyces sp.* predominate the fungal isolates as they took 17.65% each of the total isolates while *A. fumigatus* took 11.76% and the other isolates 5.88% each.

The results of the study also showed that temperature is an important factor which influences the growth rate and development of the fungi isolated. Though both batches of the culture plates (Batch A at 25° C and Batch B at 37°C) showed growth with a similar spectrum of organisms isolated at both temperatures, it was observed that culture plates incubated at 37°C supported faster growth of the fungal isolates.

Also of importance is the pH of the dried Ogi, which was found to be in the acidic region as indicated on Table 3.

The result of the ecological parameters are represented on Tables 3.

TABLE 3: ECOLOGICAL PARAMETERS OF THE DRIED OGI

SAMPLE	pH	TEXTURE	COLOUR	% Moisture Content
Dried Ogi	4.85	Rough	Off white	26.23

DISCUSSION

The study has shown that there is a wide spectrum of fungal species associated with dried Ogi. These organisms gain entry into the dried Ogi through a variety of means and tend to grow and develop depending on the presence of favourable conditions, which support their growth. Of the 10 genera of fungi isolated, *Aspergillus niger, Penicillium sp.* and *Saccharomyces sp,* had the highest occurrence, been isolated on both media used. The genus *Aspergillus* is a micro flora of maize grain (Banwart, 1974). Almost all the fungi isolated have been reported in maize and maize products (Frazier and Westhoff, 1988).

Akinrele (1970) reported that Ogi contains carbohydrates, some amino acids such as Serine, Lysine, Tryptophan and Glutamic acid and that it also contains some vitamins e.g. Riboflavin, Niacin, and Thiamine and these serve as the source of nutrients to meet the nutritional needs of the fungi in the Ogi, thus facilitating their colonization and survival in the dried Ogi.

Also, the pH of the dried Ogi was determined as 4.85. This is acidic and is due to the fermentation process which led to accumulation of some acids e.g. Lactic and Carboxylic acids. Frazier and Westhoff (1988) reported that the growth of majority of fungi is favoured by an acid pH and this may have been a factor which contributed to the large number of fungal isolates in the dried Ogi. The same authors also reported that the

approximate limiting total moisture content of a given food for fungi growth is below 14-15%, hence any percentage above this will support fungi growth. Since the percentage water content in the Ogi was determined as 26.25%, it indicates that the water content of the Ogi was adequate to support its colonization by the fungal isolates.

Another source of contamination could be the drying process, which is usually done in the open, under the sun, and this exposes the Ogi to contamination by air borne spores and the natural fungal flora of the environment. It is important to note that the specific type of fungi found in the dried Ogi may be influenced by the type of fungal species which are indigenous to the area where drying is carried out. Another possible source of the fungal isolates is the residual organisms from the fermentation process. Some of the fungi isolated are those involved in the fermentative production of Ogi e.g. *Saccharomyces sp., Fusarium sp., Aspergillus sp.* etc (Jay, 2004). Lastly, it is also possible that the fungal isolates may have gotten into the dried Ogi during the production process from humans involved, who may have been carrying fungal spores on their clothes, hair etc or from inanimate objects such as sieves, stirrer or containers used during the process.

It is known in medical science that a large percentage of tropical diseases are caused by fungi. Also some of the fungal species isolated from the dried ogi have the capacity to produce mycotoxins and some of these toxins have been shown to be mutagenic, carcinogenic and can cause specific organ toxicity e.g. hepatotoxicity as mentioned earlier. However, NOT all strains of these fungal species have the ability to produce mycotoxins. Also, some of the fungal species isolated from the dried Ogi also have the ability to colonize and cause invasive disease in humans e.g. Aspergillosis, which is caused by *Aspergillus fumigatus* and other *Aspergillus* species (Mitchell, 2001). The same author also reported that Penicilliosis may be caused by *Penicillium marneffei* and Mucormycosis (Zygomycosis) caused by some species of *Mucor* and other Zygomycetes. The incidence of disease caused by fungi is greatly increased in immunocompromised individuals such as AIDS patients (Peddler, 2004).

Since Ogi is a widely consumed food product, it is recommended that industrial processes be adapted for its production as this will lead to improvement in the quality of the product through the employment of Good Manufacturing Practices (GMP) and other quality control standards. Also, since the microorganisms involved in the fermentation process of Ogi have been identified, they can be specifically isolated and genetically improved so that they can be used in its industrial production. The use of automated equipment in the industrial manufacture of Ogi will eliminate human beings as a source of contamination and also, the use of equipment such as solar dryers in the drying process will eliminate contamination through air spora. Public enlightenment campaigns can also be undertaken to enlighten the public on the impact of these contaminants on their health and thus advice them on safety measures which they can employ such as improved hygiene during the production process to exclude some of these contaminants. Also, nursing mothers using ogi as a weaning food should be advised not to wean their infants at an early age (3-6 months as commonly occurs) but to continue breastfeeding until the WHO recommended age of 2years, when the child's immune system would have been considerably developed to fight infections.

CONCLUSION

From the observations made and the results obtained in the study, it has been shown that several species of fungi are associated with dried Ogi due to the favourable conditions which it provides for fungal growth. Some of these fungi have the potential to cause disease and some can produce poisonous substances known as mycotoxins. Some steps which can be taken to counter this problem such as encouraging the use of facilities such as solar dryers in drying the Ogi, have also been proposed.

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Correspondence author:
U. Ajima
Department of Pharmaceutical Chemistry, University of Jos, Jos, Nigeria.
Email: descar84@yahoo.com